PHYLOGENETIC RELATIONSHIP OF LECTIN-LIKE PROTEINS EXPRESSED IN TEPARY AND COMMON BEAN

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Pulses contain seed storage proteins that deter insect predation of the grain. In particular, lectin family phytohaemagglutinins (PHA), α-amylase inhibitors (α-AI), trypsin inhibitors, and arcelins (ARL) are involved. PHAs and α-AIs show mammalian toxicity whereas ARLs and to some extent, α-AIs, seem specific to insect predation, mainly seed weevils or bruchids. Arcelin belongs to the APA (ARL-PHA-α-AI) complex locus. Seven alleles for arcelin have been discovered in wild accessions of common bean, and some of these have been incorporated into cultivated material. Some alleles provide high levels of resistance to the bean bruchid *Zabrotes. subfasciatus*, but none provide more than moderate levels of resistance to *Acanthoscelides obtectus*. Until recently, it was not known whether arcelins occur in *Phaseolus* species other than *P. vulgaris*, but the presence of these compounds in tepary bean (*P. acutifolius*) was suspected. Researchers at CIAT identified G40199, a wild tepary accession that is highly resistant to both bruchid species. The objective of our research was to transfer resistance to common bean and determine if arcelin was involved.

MATERIALS AND METHODS

Germplasm consisted of G40199, 'Brown Tepary', a cultivated tepary maintained at OSU, 'ICA Pijao', a small seeded tropical black that is cross compatible with tepary bean, and 'Rojo', a large red-seeded cultivar developed by Sokoine University of Agriculture in Morogoro, Tanzania. Interspecific hybrids were obtained from crosses between G40199 and ICA Pijao as described previously (Kusolwa & Myers, 2005). A candidate gene approach was used to identify sequences of the lectin-like proteins in G40199 and derivatives. Primers were designed from APA locus sequences of *P. acutifolius* deposited in Genbank and used to amplify PCR fragments from genomic and cDNA in the target genotypes. Bands of interest were excised from gels and sequenced. DNA sequence was translated into coding amino acid sequence for phylogeny studies. Sequences were compared to existing common bean and tepary sequences obtained from NCBI and EMBL databases. Neighbor joining algorithm in PAUP with 1000 bootstrap repetitions was implemented to obtain a phylogenetic tree. Sequences were aligned to further examine evolutionary relationships.

RESULTS AND DISCUSSION

G40199 was initially examined to determine its seed storage protein profile. A novel 33 kDa band was observed on SDS-PAGE protein gels in G40199, but not in cultivated tepary bean or common bean. The observed protein band was similar in size to that expected for arcelin of common bean and cosegregated with arcelin as revealed in genomic DNA. Using primers designed from previously published arcelin-like gene sequences from *P. acutifolius*, we demonstrated that both G40199 and Brown Tepary had putative arcelin proteins. Protein peptides sequencing (Kusolwa, 2007) confirmed that these proteins are functional arcelins.

Particularly surprising was the observation that G40199 possesses two separate genes for arcelin. These, as part of the complete APA locus found in this accession, may be the reason that it possesses such strong resistance against both major bruchid species of beans. We do not know how the two arcelin genes are physically arranged, but based on the work of Kami et al. (2006), would predict

that they lie in tandem with the other genes of the APA locus. The two genes are quite divergent, with one showing greatest similarity to the arcelin found in cultivated tepary, whereas the other appears unique to the wild accession.

We conducted a phylogenetic analysis of the novel lectin-like proteins observed in the tepary bean accessions used in the present study. Our neighbor joining tree for *P. vulgaris* was very similar to that presented by Lioli et al. (2003). The phylogeny fits well with the hypothesis that PHA is ancestral to α-AI and ARL, and that the latter two were derived independently from PHA. Our data suggests that arcelins and α-AI were both derived prior to the separation of *P. vulgaris* and *P. acutifolius* as species. Also, α-AI alleles from the two species appear to have diverged less than the arcelins. It is not possible to determine whether the rate of change in α-AI has been slower than in the arcelins, or whether arcelins are more ancient than α-AIs.

When sequences are aligned, three gaps were observed that characterize the phylogenetic relationships among the seed proteins (Mirkov et al., 1994). GAP 1 is associated with α-AIs of both species. GAP 2 occurs in all *P. acutifolius* arcelin sequences and *P. vulgaris* α-AI-2 (the latter of different size and probably being derived independently). GAP 3 separates PHA from the lectin-like proteins. Temporally, GAP 3 probably occurred first, GAP 1 next, with GAP 2 being the most recent event.

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